Effects of Extremely Low Frequency (ELF) Electromagnetic Fields on the Diatom Community of the Ford River, Michigan

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Abstract

The effects of 76 Hz extremely low frequency (ELF) electromagnetic radiation produced by the U.S. Navy's ELF andtenna on riverine diatom communities have been intensively studied since 1983 at two sites in the fourth order Ford River in northern Michigan. Data from a control site were compared to data from an experimental site under the antenna. Background data on the diatom community were collected from June 1983 through April 1986; transitional data were collected on ELF effects during a variable power testing period from May 1986 until October, 1989; and data from a fully operational system were collected from October 1989 through September 1990. Diatoms were monitored at a site near the antenna and at a control site where they received 5.5 times less exposure to longitudinal electric fields and 330 times less exposure to magnetic flux than they received at the antenna site. Paired t-test analyses between sites showed that chlorophyll a and organic matter accrual rates were different between the sites in 1990, the first year of fully operational ELF exposure, whereas they were not different when data from 1983 through 1990 were compared. Before and after, control and impact analyses (BACI) and randomized intervention analyses (RIA) suggested that the relationship between sites had changed for chlorophyll a biomass, diatom cell density, total diatom biovolume, and species diversity and evenness after ELF testing began as compared to data collected prior to such testing indicating a possible ELF effect. Chlorophyll *a* gave the strongest evidence for an ELF effect of any of the 9 community based algal parameters examinded. Analysis of covariance (ANCOVA) with longitudinal electric field included as a covariant suggested that this type of electric field was not responsible for the changes observed through 1989 (1990 exposure data were not available at the time of this analysis). Ongoing analyses are being conducted to determine if observed changes are related to the greater exposures to longitudinal fields experienced by the algae in 1990 or to changes in magnetic flux or to weather related variables such as water temperature. Stepwise regressions indicated that water temperature was the most important of the weather related variables in explaining variance in diatom community data. We also examined ELF effects on 3 of the most common species of diatoms present in the summer and 4 of the most common species present in the winter. The abundance of none of these species changed between the before and after data sets providing no evidence for ELF effects at the individual population level.

Key Words: Diatoms, ELF electromagnetic radiation, rivers, diversity, density, species composition, chlorophyll *a*.

Introduction
In 1982, a study to determine the effects of extremely low frequency (ELF) electromagnetic radiation on diatom (Division Bacillariophyta: Class Bacillariophyceae) (classification after Patrick and Reimer 1966) communities in the Ford River, Michigan was initiated. The ELF electromagnetic radiation was to be produced by a 56 mile long antenna (one 28 mile NS leg and two 14 mile long EW legs that crossed the NS leg) to be built by the U.S. Navy for communication with deeply submerged submarines. The initial subcontract with IIT Research Institute (funded as part of their contract with the U.S. Navy) called for three years of before and three years of after data for determining ELF effects at paired sites with one located under the site where the antenna was to be built (experimental site) and the other far enough away so that ELF electromagnetic radiation would be at least an order of magnitude less than the radiation received by organisms under the antenna (control or reference site). After 10 months of selecting preliminary sites and collecting data on the biota, chemistry, and physical characteristics of the sites, and on the ambient ELF fields (collected by IITRI) at the sites to make sure that the final sites selected were as comparable as possible, background data collection began at the two selected sites. Background or before data collection continued from June, 1983 to May, 1986; transitional data were collected from May, 1986 to October, 1989; and fully operational data have been collected since October, 1989. The transitional data were collected during a period of intermittent antenna testing that consisted of only 35 days of testing for the NS leg that crossed the aquatic sites in 1986 (75 days for all legs) at 4-6 amps; limited exposure at 15 amps in 1987; limited exposure at 75 amps in 1988; intermittent exposure at 150 amps in most of 1989 before going operational in October, 1989 at 150 amps and 76 Hz. This paper includes data on the effects of ELF fields on the benthic algal community through early September, 1990. The objective of this paper is to summarize our results on ELF effects on the diatom community in the Ford River. To our knowledge, no other studies of ELF effects on natural, in situ algal communities have been conducted.

Methods and Materials
The two sites chosen for the study of ELF effects were in riffle zones of the fourth order Ford River in northern Dickinson County in Michigan's upper peninsula. The Ford River is part of the Lake Michigan drainage. The Ford River catchment lies between the Escanaba and Menominee River catchments, and the river empties into upper Green Bay just south of Escanaba, Michigan. At the two study sites, the stream varies from 9-12 m wide during low flow, and depth varies from less than 0.3 m during low flow to more than 2 m during floods. Chemical and physical data for the river have been extensively monitored, and preliminary data are available from Burton et al. (1991 a, b) and Oemke and Burton (1986). The river can be characterized as a hardwater, high alkalinity (about 120 - 180 mg $CaCO_3$/L), low nutrient (less than 10 μg soluble reactive P/L and less than 60 μg inorganic N/L), brown water trout stream with a bottom that varies from sand and silt in the pools to gravel to cobble in the riffles. Most of the land in the catchment is in successional forest with *Populus tremuloides* dominating the upland areas and *P. balsamifera* and/or *Alnus rugosa* dominating riparian zones. Extensive *Alnus rugosa* and some *Thuja occidentalis* swamps in the drainage basin along with forest humus and litter are likely sources of the organic matter that imparts the brown color to the river. The pH in the river varies between 7.5 and 8.2 most of the time, and dissolved oxygen remains near saturation throughout the year.

The experimental site was located within 50 m of the point where the ELF antenna crossed the Ford River (T43N:R29W:Sec. 14), while the control or reference site was about 8 km downstream (T43N:R28W:Sec. 21). These two sites were closely matched in exposure to ambient ELF electromagnetic levels prior to operation of the antenna. At full power in 1989, 5.5->10 times more ELF radiation was received by the biota at the experimental site than at the control site (e.g. the experimental site received 5.5 times more exposure to longitudinal electric fields; 540 times more exposure to transverse electric fields, and 330 times more exposure to magnetic flux than did the control site at 76 Hz according to measurements made by IITRI in 1989). At full power of 150 amps, biota at the experimental site received ELF exposure at a rate of 61 mv/m^2. Placement of samplers in 1990 was adjusted to insure that biota at the experimental site received at least 10 times more exposure for all components of ELF electromagnetic radiation measured at 76 Hz during full power operation than did the biota at the control sites.

Standard (25X76 mm), glass microscope slides were held vertically into the current in plexiglass diatom racks using a modification of the diatometer illustrated in Patrick and Reimer (1966) except that no deflector shield was placed in front of the slide holder, and the racks were fastened directly to standard construction style clay bricks that were placed directly on the stream bottom. The slide racks were placed in riffle areas, and current velocity through the slide holders was carefully matched using a current meter. Positions of the racks were checked and adjusted weekly to insure that current velocity differed little between sites. Care was also taken to place the racks in areas of the stream that were open to sunlight during most of the day. Some early morning and late afternoon shading by riparian vegetation and/or banks of the streams was unavoidable but shading differences between sites was minimal.

Colonization times of 28 days for the mature community and 14 days for chlorophyll a accrual rates during the ice-free seasons were selected based on the findings of Oemke and Burton (1986) for this river during start up studies in 1982. Winter sampling occurred at 28 day intervals from 1983-84 through 1986-87, at 56-60 day intervals in 1987-88, and has remained at 42 day intervals since that time. Processing of diatoms for counting, calculations, and other procedures followed the methods discussed by Oemke and Burton (1986) as did procedures for determining chlorophyll a and organic matter accrual rates and biomass standing crops. We use accrual rates as crude estimates of primary productivity as recommended in Standard Methods (A.P.H.A. 1985). We prefer to refer to these measures as accrual rates, since productivity is underestimated or affected by an unknown amount due to sloughing losses, leakage of cellular contents, death and senescence of cells, etc.

All statistical procedures except for before and after, control and impact (BACI) analyses and random intervention analyses (RIA) were performed using StatView 512+ (copyright 1986 by Abacus Concepts, Inc.), a software program for the Apple Macintosh plus from Brainpower, Inc. of Calabasas, CA. Procedures for BACI and RIA analyses are summarized in Eggert et al. (1991), a companion paper in this volume. Differences between treatments were accepted as significant at the $p>0.05$ level unless otherwise specified.

Results

There were no significant differences for any of the nine community level benthic algal parameters monitored at the antenna and control sites from 1983 through 1990 according to paired t-tests analyses (Table 1). Values at the control site and the antenna site were significantly correlated for each of these parameters (Table 1). Minimum detectable differences were in the 25-30 % range for most parameters with Shannon-Wiener diversity (H') and evenness (J') being the most sensitive indicators for detection of differences between the sites (5-7 % - Table 1). Diatom cell density and total diatom biovolume (calculated from individual cell volumes and density) were the least sensitive parameters for detection of differences between the sites (48 -53 % minimum detectable differences - Table 1).

Table 1. Paired t-test results (DF = 83 - 85), correlation coefficients*, and percent minimum detectable differences ($p < 0.05$) between the antenna and control sites for benthic algal parameters for 1983 - 1990.

Parameter	t-test	correlation coefficient	minimum detectable difference
Chlorophyll a Biomass	NS	0.85	29 %
Chlorophyll a Accrual	NS	0.83	32 %
Organic Matter Biomass	NS	0.70	23 %
Organic Matter Accrual	NS	0.61	27 %
Diatom Cell Density	NS	0.90	48 %
Diatom Cell Volume	NS	0.96	25 %
Total Diatom Biovolume	NS	0.70	53 %
Species Diversity	NS	0.70	7 %
Species Evenness	NS	0.79	5 %

* all are significant at the $p < 0.01$ level.

We also compared the antennna to the control site for each year of the study using the paired t-tests. Few differences were detected on a year by year basis. The first fully operational year for ELF exposure was 1990. Comparisons between the antenna and control sites for 1990 using paired t-test analyses suggested that two of the parameters listed in Table 1, chlorophyll *a* biomass and daily accrual rates of organic matter, had been affected by ELF exposure. Chlorophyll *a* biomass was slightly but significantly higher under the antenna than it was at the control site. However, chlorophyll *a* daily accrual rates were not significantly different between the sites in 1990. Conversely, organic matter biomass was not signifcantly diffferent between the two sites in 1990 even though organic matter daily accrual rates were. Since algal and bacterial biomass should dominate production of organic matter on slides oriented vertically to the current (little settling of suspended organic matter should occur in this orientation), it is surprising that results from the two parameters are not more closely correlated. No significant differences for any of the other seven community based parameters listed in Table 1 were detected in 1990. No significant differences had occurred between sites overall (Table 1) or even for 1989, the year of intermittent exposure to 150 amp operation of the antenna. Significant results should occur by chance alone 5 % of the time. With two of the nine community based parameters (22 %) being significantly different between the sites in 1990, differences appear to be real. We feel that it is too early to put much emphasis on these analyses after only a single year of fully operational data and will

Table 2. Summary BACI and RIA statistics. Before statistics are from June 1983 to April 1986. After statistics are from May 1986 to September 1990. N in parentheses for BACI and RIA respectively. NS = $p > 0.05$. N = 83 - 85 overall, 48 - 53 for summer, 33 - 35 for winter samples.

Parameter	BACI	RIA
Chlorophyll a Biomass	$p < 0.01$	$p < 0.05$
Summer Data Only	$p = 0.06$	$p < 0.01$
Winter Data Only	NS	NS
Organic Matter Biomass	NS	NS
Summer Data Only	$p < 0.01$	$p < 0.01$
Winter Data Only	NS	NS
Diatom Cell Density	$p < 0.01$	$p = 0.06$
Summer Data Only	$p < 0.01$	$p = 0.18$
Winter Data Only	NS	NS
Diatom Cell Volume	NS	NS
Summer Data Only	NS	NS
Winter Data Only	NS	NS
Total Diatom Biovolume	$p < 0.05$	$p = 0.09$
Summer Data Only	NS	NS
Winter Data Only	NS	NS
Species Diversity	$p < 0.05$	$p < 0.05$
Summer Data Only	NS	NS
Winter Data Only	NS	NS
Species Evenness	$p < 0.01$	$p < 0.01$
Summer Data Only	$p < 0.05$	$p < 0.05$
Winter Data Only	$p < 0.05$	$p < 0.05$

await the 1991 data before drawing firm conclusions.

Studies that use comparisons between a single control and reference site have a potential problem with pseudoreplication (Stewart-Oaten et al. 1986, Carpenter et al. 1989), and there is some question as to whether or not paired t-tests are appropriate ways to analyze the data. The two statistical procedures suggested as alternatives by Stewart-Oaten et al. (1986) and Carpenter et al. (1989) to the more traditional paired t-test approach have been used on our data as an additional way to detect potential differences between the two sites that may be related to ELF electromagnetic fields (Eggert et al. 1991). Since RIA analyses require a minimum number of 40 observations, analyses are only possible at this time using all the transitional and operational data (May 1986 - September 1990). We ultimately plan to stratify results into before data (June 1983 - April 1986), transitional data with the ELF antenna undergoing testing (May 1986 -

September 1989), and operational data (October 1989 - September 1992). Both the RIA and BACI analyses test for differences in the relationship of the data between the two sites before and after a potential impact occurs. The means do not have to be the same to show no significant difference. Suppose, for example, that density had consistently been higher at the control site than at the impact site prior to the onset of the potential environmental stress (e.g ELF exposure in this study). No impact would be indicated by either analysis if this difference remained consistent after onset of the potential stress (e.g. exposure to ELF fields). If, however, the relationship changed such that mean density decreased at the impact site but not at the control site, BACI and RIA analyses would indicate a significant change even though the means might now be comparable. This significant change could signal a potential significant change in density at the impacted site related to ELF exposure (Eggert et al. 1991).

Results of the preliminary BACI analyses for 7 of the 9 community based parameters reported in Table 1 indicated that significant differences have occurred between the before and transitional/operational data for 5 of the 7 parameters examined using the overall data set (line one for each parameter in Table 2). RIA analyses confirmed these differences as being significant for 3 of the 5 parameters. Both RIA and BACI analyses showed that before data were different from transitional/operational data for chlorophyll *a* biomass and species diversity and evenness indicating a potential ELF effect on these parameters (Table 2). BACI analyses showed that before diatom cell density and total diatom biovolume data differed significantly from after data, but RIA analyses failed to confirm these results at the $p > 0.05$ level (differences were significant at the $p > 0.10$ level - Table 2). No differences were detected for any of these 5 parameters using the paired t-tests on the combined data (Table 1).

Winter data are more variable than summer data, are collected at less frequent intervals, and could mask differences in the data sets. Therefore, we stratified the data into data collected from mid-April through October (summer data) and data collected from November to April (winter) to see if we could improve results (Table 2). Of course, smaller sample size results in less power to detect differences. Even so, differences occurred in the organic matter biomass summer before and after data, that were not evident in the overall data set even though significant differences in some of other parameters disappeared (Table 2). Species evenness was the only parameter that showed a consistent difference in the winter as well as in the summer and overall (Table 2).

BACI analyses were used to compare year to year differences, since it is less sensitive to sample size than is RIA analyses. While some of the other parameters showed differences in at least one of the before years compared to at least one of the transitional/operational years, only chlorophyll *a* biomass showed a significant difference between data collected in 1990, the only fully operational year so far, and each of the before ELF years (83, 84, and 85). Since paired t-test analyses had also indicated that differences between the control and antenna site were siginifcant in 1990, chlorophyll *a* offers the best evidence that ELF electromagnetic radiation may have caused a difference in benthic algae between the two sites. Chlorophyll *a* was slightly but significantly greater at the antenna site than at the reference site in 1990. If ELF has caused an effect, it has resulted in slightly increased biomass of chlorophyll *a* at the antenna site.

Since a variety of differences were detected between the before and transitional/operational data, we analyzed the data using analysis of covariance (ANCOVA) with cumulative exposure to ELF longitudinal fields over the 28 day colonization period as the covariant for the 1986 through 1989 after data (Table 3). The 1990 ELF exposure data are not yet available. The only difference between sites detected in

Table 3. Analysis of covariance (ANCOVA) statistics for 1986 - 1990. Before and after t-test comparisons are for before period from June 1983 to April 1986 and May 1986 to September 1990 respectively. NS = $p > 0.05$.

Parameter	Between Site t-Tests Before	After	ANCOVA
Chlorophyll a Biomass	$p < 0.05$	NS	NS
Organic Matter Biomass	NS	NS	NS
Diatom Cell Density	NS	NS	NS
Diatom Cell Volume	$p < 0.05$	NS	NS
Total Diatom Biovolume	NS	NS	NS
Species Diversity	NS	$p < 0.01$	$p < 0.05$
Species Evenness	NS	$p < 0.01$	$p < 0.05$

the after data using between site t-tests were for species diversity and evenness (Table 3). ANCOVA did not change any of these relationships (Table 3) indicating that diffences between sites was not related to exposure to longitudinal electric fields from 1986 through 1989. Therefore, differences detected between sites as summarized in Tables 1 and 2 and above must be related to some factor other than ELF longitudinal electric fields or to the greater differences in longitudinal electric fields in 1990, the first year of fully operational data. Exposure to increased magnetic flux could be the explanation as could between year differential responses to weather related factors between the sites. Stepwise regression analyses strongly implicated water temperature as the most important weather related factor in explaining variance in the benthic algal community. Future analyses will explore water temperature and ELF generated magnetic flux exposure as covariants that may explain differences in the before and after data between sites. As soon as 1990 data on longitudinal electric fields are available to us, we will incorporate these data in our ANCOVA analyses.

Since BACI and RIA anayses had indicated differences in the before and transitional/ operational data in species diversity and evenness, we selected three of the more common species of the diatom flora from the summer period and four from the winter period for further analyses (Table 4). There were no significant differences for any of these species between the before and transitional/operational data (Table 4). Differences in diatom species diversity and evenness must, therefore, be related to differences in rarer species rather than these more common forms.

In summary, data collected to date on response of the benthic algal community to ELF electromagnetic fields suggest that changes may be occurring in the algal community that may be related to exposure to ELF electromagnetic fields. These changes include

Table 4. Summary BACI and RIA statistics for selected diatom species. Before statistics are from June 1983 to April 1986; N = 44-45 for summer and 31 for winter. After statistics are from May 1986 to September 1990; N = 45-46 for summer and 33 for winter. NS = $p > 0.05$.

	BACI	RIA
Summer		
Achnanthes minutissima	NS	NS
Cocconeis placentula	NS	NS
Cymbella minuta	NS	NS
Winter		
Achnanthes minutissima	NS	NS
Fragilaria vaucheriae	NS	NS
Gomphonema olivaceum	NS	NS
Synedra ulna	NS	NS

changes in chlorophyll *a* biomass, organic matter accrual rates, diatom density, and diatom species diversity and evenness. The strongest evidence for such changes comes from the chlorophyll *a* biomass data. There is no evidence to suggest that any significant changes have occurred in numbers or volume of the more common algal species in the community. Potential effects on the community cannot be completely confirmed at present due to only a single year of exposure to the fully operational ELF antenna. Studies on the benthic algal community over the next one or two years should clarify the preliminary results reported in this paper.

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